Cloud Point Extraction and Determination of Trace Iron(III) in Urine Samples by Spectrophotometry and Flame Atomic Absorption Spectrometry

AHMED FADHIL KHUDHAIR* and MOUYED KHUDHAIR HASSAN

Department of Chemistry, College of Science, University of Karbala, Karbala, Iraq

Received: 8 June 2017; Accepted: 17 September 2017; Published online: 30 October 2017; AJC-18623

Cloud point technique used effectively for extraction and pre-concentration of iron(III) in the urine samples of occupational workers prior measured by using flame atomic absorption spectrometry and UV-visible spectrophotometer. The metal responds with benzidine as reagent in a non-ionic surfactant Triton X-114 medium. The main factors affecting cloud point extraction efficiencies, such as pH of sample solution, concentration of benzidine reagent, type of surfactant, concentration of Triton X-114, effect of salt out, influence of interferences and impact of equilibration temperature and time were studied. The calibration curve was linear in the range of 0.25-3.0 µg mL⁻¹ with r² = 0.9655 for UV-visible spectrophotometer at λmax 425 nm. The limit of detection was 0.25 µg mL⁻¹. The relative standard deviation for six replicates was 3.071 %.

Keywords: Cloud point extraction, Triton X-114, UV-visible spectrophotometer, Iron(III), Benzidine, Urine samples.

INTRODUCTION

The increase in the concentration of trace components relative to the total component concentration can be achieved with pre-concentration techniques [1]. There are multiple methods of pre-concentration for metals, like solid phase extraction (SPE), cloud point extraction (CPE), electrochemical deposition, co-precipitation and precipitation, liquid-liquid extraction (LLE) and ion-exchange [2-5]. In this study, emphasis was placed on the use of cloud point extraction technique for several reasons viz., high recovery efficiency, high concentration coefficient [6], green chemistry, less consuming reagent, less production of chemical residues, surfactant used is safe, non-volatile and non-toxic simplicity [7,8]. A pre-concentration method including the process of cloud point extraction is depended on the use of non-ionic varieties of surfactants in aqueous solutions and thus the formation of micelles and by heating these solutions to a certain temperature will become cloudy or turbid this temperature is known as the cloud point temperature [9]. Cloud point extraction is used with coupling with different techniques for estimating various elements in different samples such as, cloud point extraction to pre-concentration and analysis of strontium (II) ion by turbidimetric method using Schiff base derivative [10].

The increases in the concentration of iron in the body have negative effects including endocrine problems and diseases of the liver, lung and other diseases [11-14]. The previous literature review conducted to determine the iron element in various samples by using cloud point extraction method that is coupled with different techniques such as: flame atomic absorption spectrometry (FAAS) [12,15,16], UV-visible spectrophotometer [17,18], inductively coupled plasma mass spectrometry [19], graphite furnace atomic absorption spectrometry [20] and capillary electrophoresis [21]. The urine has a pH of 5.5-7.0 and a range of 6.2 [22], while the concentration of iron element in the urine is estimated to be about the median and range concentration of iron in urine was 4.9 ng mL⁻¹ and < 2.1-16.4 ng mL⁻¹ [23-26]. The aim of this study was to develop simple spectrometric method for the determination of Fe(III), using benzidine as a complexing agent and cloud point extraction. The proposed method is successfully applied for the determination iron(III) in urine of occupational worker samples.

EXPERIMENTAL

A pH meter WTW (model 720) with a combined glass electrode was used for pH measurements. A Hettich centrifuge (model EBA-20, Germany) with 10 mL calibrated centrifuge tubes was used for phase separation at 3600 rpm for 5 min. A Lab Line Super mixer (model 129) Hitech Trader, USA was used to mix the solutions. Shimadzu double beam UV-VIS Spectrophotometer, UV-1800, Japan is used to measure all
absorption spectra. The metals ions have been determined by using flame atomic absorption spectrophotometer (FAAS) (Buck scientific model 210 VGP, USA) with deuterium background correction equipped with 10 cm of air/acetylene flame burner head and hollow-cathode lamp that can be changed with metal ion.

Most of the chemicals have been prepared by using analytical grade chemicals and deionized water. Benzidine reagent (1% w/v) was prepared by dissolving 1 g of benzidine in 1-2 mL dimethyl sulfoxide and then add absolute ethanol to 100 mL in a volumetric flask. Solutions of non-ionic surfactant Triton X-114 have prepared at 20 % (v/v) concentration by diluting 20 mL of Triton X-114 to 100 mL (hot deionized water) in a volumetric flask. A stock solution of 1000 mg/L of Fe(III) was prepared by dissolving 0.2920 g of FeCl₃ in 100 mL deionized water.

Sample collection: A 44 urine samples were collected from occupational workers workers in oil refineries and terminals engaged in dyeing, welding, plumbing, etc. The urine samples were collected in a new polyethylene bottles (120 mL) and stored at 0-5 ºC [27].

Urine sample digestion: In a 100 mL beaker, 25 mL of urine sample accurately measured, was treated with a mixture of 5 mL H₂O₂ and 2.5 mL of conc. HNO₃, and was placed on a hot plate. Then the samples are heated until the sample become complete dry. Thereafter, a dry residuum with a dark colour is added to 2.5 mL of conc. HNO₃ and heated again to the point of drought. This step is repeated several times until a white ashes were obtained. The white ashes were dissolved in the final step by using 2.5 mL of 3M of HCl [28,29].

Cloud point extraction: The precocentration and extraction procedure for iron(III) by using cloud point extraction method aliquots of 10 mL of Fe(III) solution containing 2 µg mL⁻¹ and 0.5 mL of 1 % (w/v) of benzidine reagent was mixed. The green colour solution product formed has λ max 425 nm depending on the absorption spectra of UV-visible spectroscopy for Fe(III)-benzidine complex. After adjusted the pH at 4.2 by using 0.1 M of HCl and 0.1 M of NaOH, added 0.1 mL of 20 % (v/v) Triton X-114. Then the mixture placed on the super mixer to mix and heated on a water bath at 70 ºC for 10 min. This step follows the separation of the solution into two layers via the centrifuge at 3600 rpm for 5 min. After that the phases are cooled using an ice bath at 0-5 ºC to increase the viscosity so that surfactant-rich phase separated easily by converting tube. The remaining micellar phase containing Fe(III)-benzidine complex was dissolved in 0.75 mL of absolute ethanol and transferred into 1 cm quartz cell (1 mL). Then measured the absorbance of solution against a blank solution prepared in the same way. The proposed reaction mechanism for Fe(III)-benzidine complex formation is shown in Fig. 1.

![Fig. 1. Proposed reaction mechanism for Fe(III)-benzidine complex formation](image-url)
Absorption spectra for Fe(III)-benzidine complex: The absorbance spectra for benzidine, iron(III) solution and Fe(III)-benzidine complex in surfactant rich phase are demonstrated in Fig. 2. The absorbance spectra of benzidine reagent shows $\lambda_{\text{max}}$ 288 nm, iron(III) solution has $\lambda_{\text{max}}$ 320 nm, while Fe(III)-benzidine complex has 425 nm.

RESULTS AND DISCUSSION

The optimal conditions for high efficiency of cloud extraction were studied and stabilized. The most important conditions are order addition, pH, concentration of reagent, types of surfactant, concentration of surfactant, equilibrium temperature, time of incubation, salt out effect and interfering ions.

Order addition effect: The most important factors that have been studied are order additions where it changed the order of (2 $\mu$g mL$^{-1}$, 10 mL) of Fe(III) and (1% w/v, 0.5 mL) of benzidine reagent (Table-1). The greatest absorbance value was chosen accordingly.

<table>
<thead>
<tr>
<th>Type of complex</th>
<th>Order addition</th>
<th>Absorbance of an aqueous layer (Aq)</th>
<th>Absorbance of a rich phase surfactant (As)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe(III) complex</td>
<td>M + R + T</td>
<td>0.000</td>
<td>0.295</td>
</tr>
<tr>
<td></td>
<td>R + M + T</td>
<td>0.000</td>
<td>0.301</td>
</tr>
</tbody>
</table>

Effect of pH: pH is one of the most important factors that directly affect the formation of the iron complex by using cloud point extraction. The test was applied using the range of pH 2.7-10.4. These values were adjusted using by 0.1 M NaOH and 0.1 M HCl solution. The absorbance increased, when the pH value equal 4.2, the complexation reaction at pH values lower than 4.2 is incomplete probably due to the protonation of benzidine (Fig. 3). On the other hand at pH value higher than 4.2 is due to hydrolysis of Fe(III). The effects of different types of acids (0.1 M) are shown (Fig. 4). The effect of hydrochloric acid on Fe(III)-benzidine complex formation is stronger as compared to other acids, where it gave the highest absorbance. It is also found that volume 0.75 mL of 0.1 M of HCl gave best absorbance (Table-2).

![Fig. 2A. Absorption spectra of iron(III) solution](image1)

![Fig. 2B. Absorption spectra of benzidine reagent solution](image2)

![Fig. 2C. Absorption spectra of iron(III)-benzidine complex](image3)

![Fig. 2D. Over lapping for Fe(III)-benzidine complex](image4)

![Fig. 3. Effect of pH](image5)

![TABLE-1 EFFECT OF ORDER ADDITION](image6)

![TABLE-2 EFFECT CONCENTRATION OF HCl](image7)
Effect of reagent concentration: The concentration effect of chelating agent i.e., benzidine on absorbance value was studied by using various volumes from 0.05 to 1.00 mL of 1% w/v of benzidine while keeping other factors constant. The results (Fig. 5) show that the absorbance value increased with increasing amount of added reagent benzidine. The best result was relied upon when the amount of reagent is 0.5 mL.

Influence of type and concentration of surfactant: The efficiency of cloud point extraction dependent on the type of surfactant used. Fig. 6 shows the effect type of surfactants (Triton X-100, sodium dodecyl sulfate (SDS) and Triton X-114) and its concentration on absorbance of Fe(III)-benzidine complex. Fig. 7 shows the absorbance of the complex increased by increasing the volume of 20% (v/v) Triton X-114.

Effect of equilibrium temperature and time: Fig. 8 shows the highest absorbance signal at 70 °C. In order to achieve easy phase separation and efficient pre-concentration in cloud point extraction processes, it was desirable to employ the shortest incubation time. The effect of incubation time was investigated in the ranges 1-25 min. The results demonstrate that in the incubation time of 5 min was chosen for further experiments. Fig. 9 showed the effect of incubation time on the complex formation.

Effect of salts: The effect of salts on cloud point extraction were studied by added 0.5 mL of 15% w/v of NaCl, KCl, Na₂CO₃ and Na₂SO₄ to solution of Fe(III). The results demons-
trate that the presence of some salts lead to increase the absorbance value which is attributed due to the increase in cloud point extraction efficiency since these salts will pull water molecules and destroy the hydration shell of iron ion which leads to increase the bonding between the reagent benzidine and iron. Table-3 shows the effect of salt out on complex.

Table-3

<table>
<thead>
<tr>
<th>Salts 0.5 mL of 15 % (w/v)</th>
<th>Absorbance of a rich phase</th>
<th>Recovery (%)</th>
<th>E&lt;sub&gt;a&lt;/sub&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>0.442</td>
<td>39.21917</td>
<td>60.780</td>
</tr>
<tr>
<td>KCl</td>
<td>0.401</td>
<td>35.58119</td>
<td>64.418</td>
</tr>
<tr>
<td>Na&lt;sub&gt;2&lt;/sub&gt;CO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.404</td>
<td>35.84738</td>
<td>64.152</td>
</tr>
<tr>
<td>Na&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>2.103</td>
<td>186.6016</td>
<td>86.601</td>
</tr>
</tbody>
</table>

Effect of interference ions: The effects of foreign ions on the extraction of 2 µg mL<sup>-1</sup> of Fe(III) were studied, cations that may react with benzidine or species may react with analytes and decrease the extraction efficiency to perform this study, 100 µg mL<sup>-1</sup> of interfering ions were added to a solution of 2 µg mL<sup>-1</sup> of Fe (III) and were subjected to the complete procedure. The results demonstrate that the presence of large amounts of species commonly present in water samples have significant effect on the cloud point extraction efficiency. Table-4 shows the effect of interferences on the complex.

Table-4

<table>
<thead>
<tr>
<th>Interfering ion</th>
<th>Added as</th>
<th>Concentration of Interfering ion ppm/Conc. of Cu(II) ppm</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co(II)</td>
<td>Co(NO&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>50</td>
<td>1.921</td>
</tr>
<tr>
<td>Ni(II)</td>
<td>Ni(NO&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>50</td>
<td>1.642</td>
</tr>
<tr>
<td>Cu(II)</td>
<td>Cu(NO&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;:3H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>50</td>
<td>1.803</td>
</tr>
<tr>
<td>Cr(III)</td>
<td>Cr(NO&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;3&lt;/sub&gt;</td>
<td>50</td>
<td>0.972</td>
</tr>
<tr>
<td>Al(III)</td>
<td>Al(NO&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;3&lt;/sub&gt;:9H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>50</td>
<td>0.721</td>
</tr>
<tr>
<td>Pb(II)</td>
<td>Pb(NO&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>50</td>
<td>1.721</td>
</tr>
<tr>
<td>Zn(II)</td>
<td>ZnCl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>50</td>
<td>1.812</td>
</tr>
</tbody>
</table>

Stoichiometry of the complex: The stoichiometry of the complex determined by using two methods:

**Job’s method:** In Job method, solution of reagent benzidine and iron(III) ion 1 × 10<sup>-3</sup> M were prepared and mixed in continuous variation then diluted with 10 mL of deionized water after that measured the absorbance of solution by UV-visible spectrophotometer at 425 nm. The stoichiometry of the complex determined Job’s method was found to be 2.33:1. Fig. 11 showed the Job’s ratio plot of benzidine-Fe(III) complex.

**Mole ratio method:** In the mole ratio method, fixed the volume of Fe<sup>3+</sup> ion (1 × 10<sup>-3</sup> M) and change volume of reagent benzidine (1 × 10<sup>-3</sup> M) or vice-versa, then diluted with 10 mL of deionized water and finally measured the absorbance of the solution by UV-visible spectrophotometer at 425 nm. The stoichiometry of the complex determined by mole ratio method was found to be 2.33:1. Fig. 11 showed the mole ratio plot of benzidine-Fe(III) complex.

Calibration curve of Fe(III) by UV-visible with cloud point extraction: Calibration graphs were established by plotting absorbance versus concentration of Fe<sup>3+</sup> ion. A series 0.1-7 µg mL<sup>-1</sup> Fe<sup>3+</sup> ion were prepared. Table-5 and Fig. 12 showed the range of concentration of Fe(III), results of absorbance for linear regression analysis using cloud point extraction and calibration plot for Fe(III).

**Repeatability:** This test is applied to the concentrations (0.5 and 2.0) µg mL<sup>-1</sup> of Fe(III). The values of relative standard deviation (RSD %) for Fe<sup>3+</sup> ion is tabulated in Table-6. The percentage relative standard deviation less than 5 % can be achieved using this method.
Calibration curve of Fe(III) by FAAS: A series of standard iron(III) solutions ranging from 0-5 µg mL\(^{-1}\) were used at \(\lambda_{\text{max}}\) 248.3 in order to determine the calibration curve for Fe(III) in FAAS technique. Fig. 13 shows the range of concentration of Fe(III), results of absorbance for linear regression analysis using FAAS.

Analytical applications: After the digestion of urine sample pre-concentrated by cloud point extraction and determine the Fe(III) concentration in urine of 44 samples of occupational workers by two methods UV-visible-CPE and FAAS-CPE at the optimum conditions. The results are presented in Tables 7 and 8.

Statistical treatment of results: By applying a Grubbs-test to outlier of the sample concentrations after estimating by UV-visible spectrophotometry, FAAS techniques, the results were excluded as shown in Table-9. This test was applied to 44 samples as shown in Table-10. The significance tests described so far are used for comparing means, and hence for detecting systematic errors. The F-test was used to compare the variances between the concentration of iron by two methods UV-Vis-CPE and FAAS-CPE to appear have significant or not between them [30]. Table-11 shows the results of applied F-test. And the comparison of a sample mean with true mean concentration of Fe(III) in urine samples by two methods.
TABLE-8

RESULT OF Fe(III) CONCENTRATION IN URINE SAMPLE BY USING FAAS

<table>
<thead>
<tr>
<th>ID</th>
<th>Gender</th>
<th>Age</th>
<th>Type of occupational worker</th>
<th>Duration of work (year)</th>
<th>Absorbance of Fe(III) by FAAS</th>
<th>Concentration of Fe(III) µg mL⁻¹ in urine samples by FAAS-CPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>H001</td>
<td>Male</td>
<td>55</td>
<td>Oil refineries</td>
<td>35</td>
<td>0.044</td>
<td>0.052083</td>
</tr>
<tr>
<td>H002</td>
<td>Male</td>
<td>37</td>
<td>Oil refineries</td>
<td>35</td>
<td>0.002</td>
<td>0.104166</td>
</tr>
<tr>
<td>H003</td>
<td>Male</td>
<td>28</td>
<td>Oil refineries</td>
<td>35</td>
<td>0.009</td>
<td>0.023148</td>
</tr>
<tr>
<td>H004</td>
<td>Male</td>
<td>26</td>
<td>Welding</td>
<td>2</td>
<td>0.007</td>
<td>0.029761</td>
</tr>
<tr>
<td>H005</td>
<td>Male</td>
<td>27</td>
<td>Welding</td>
<td>6</td>
<td>0.003</td>
<td>0.069444</td>
</tr>
<tr>
<td>H006</td>
<td>Male</td>
<td>50</td>
<td>Welding</td>
<td>25</td>
<td>0.003</td>
<td>0.069444</td>
</tr>
<tr>
<td>H007</td>
<td>Male</td>
<td>23</td>
<td>Plumbing</td>
<td>8</td>
<td>0.008</td>
<td>0.026041</td>
</tr>
<tr>
<td>H008</td>
<td>Male</td>
<td>22</td>
<td>Plumbing</td>
<td>7</td>
<td>0.005</td>
<td>0.041666</td>
</tr>
<tr>
<td>H009</td>
<td>Male</td>
<td>60</td>
<td>Plumbing</td>
<td>20</td>
<td>0.005</td>
<td>0.041666</td>
</tr>
<tr>
<td>H010</td>
<td>Male</td>
<td>49</td>
<td>Plumbing</td>
<td>35</td>
<td>0.005</td>
<td>0.041666</td>
</tr>
<tr>
<td>H011</td>
<td>Male</td>
<td>28</td>
<td>Plumbing</td>
<td>2</td>
<td>0.005</td>
<td>0.041666</td>
</tr>
<tr>
<td>H012</td>
<td>Male</td>
<td>29</td>
<td>Welding</td>
<td>15</td>
<td>0.004</td>
<td>0.052083</td>
</tr>
<tr>
<td>H013</td>
<td>Male</td>
<td>53</td>
<td>Dyeing</td>
<td>10</td>
<td>0.001</td>
<td>0.208333</td>
</tr>
<tr>
<td>H014</td>
<td>Male</td>
<td>45</td>
<td>Welding</td>
<td>20</td>
<td>0.006</td>
<td>0.034722</td>
</tr>
<tr>
<td>H015</td>
<td>Male</td>
<td>32</td>
<td>Dyeing</td>
<td>9</td>
<td>0.004</td>
<td>0.052083</td>
</tr>
<tr>
<td>H016</td>
<td>Male</td>
<td>25</td>
<td>Dyeing</td>
<td>11</td>
<td>0.004</td>
<td>0.052083</td>
</tr>
<tr>
<td>H017</td>
<td>Male</td>
<td>27</td>
<td>Oil refineries</td>
<td>10</td>
<td>0.004</td>
<td>0.052083</td>
</tr>
<tr>
<td>H018</td>
<td>Male</td>
<td>26</td>
<td>Dyeing</td>
<td>2</td>
<td>0.005</td>
<td>0.041666</td>
</tr>
<tr>
<td>H019</td>
<td>Male</td>
<td>35</td>
<td>Dyeing</td>
<td>20</td>
<td>0.002</td>
<td>0.104166</td>
</tr>
<tr>
<td>H020</td>
<td>Male</td>
<td>45</td>
<td>Oil refineries</td>
<td>4</td>
<td>0.003</td>
<td>0.069444</td>
</tr>
<tr>
<td>H021</td>
<td>Male</td>
<td>24</td>
<td>Welding</td>
<td>3</td>
<td>0.004</td>
<td>0.052083</td>
</tr>
<tr>
<td>H022</td>
<td>Male</td>
<td>22</td>
<td>Welding</td>
<td>8</td>
<td>0.005</td>
<td>0.041666</td>
</tr>
<tr>
<td>H023</td>
<td>Male</td>
<td>35</td>
<td>Welding</td>
<td>10</td>
<td>0.002</td>
<td>0.104166</td>
</tr>
<tr>
<td>H024</td>
<td>Male</td>
<td>42</td>
<td>Oil refineries</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H025</td>
<td>Male</td>
<td>35</td>
<td>Welding</td>
<td>25</td>
<td>0.010</td>
<td>0.020833</td>
</tr>
<tr>
<td>H026</td>
<td>Male</td>
<td>25</td>
<td>Welding</td>
<td>10</td>
<td>0.005</td>
<td>0.041666</td>
</tr>
<tr>
<td>H027</td>
<td>Male</td>
<td>38</td>
<td>Welding</td>
<td>2</td>
<td>0.005</td>
<td>0.041666</td>
</tr>
<tr>
<td>H028</td>
<td>Male</td>
<td>55</td>
<td>Oil refineries</td>
<td>12</td>
<td>0.005</td>
<td>0.041666</td>
</tr>
<tr>
<td>H029</td>
<td>Male</td>
<td>47</td>
<td>Terminals to provide fuel</td>
<td>11</td>
<td>0.004</td>
<td>0.052083</td>
</tr>
<tr>
<td>H030</td>
<td>Male</td>
<td>45</td>
<td>Oil refineries</td>
<td>10</td>
<td>0.006</td>
<td>0.034722</td>
</tr>
<tr>
<td>H031</td>
<td>Male</td>
<td>38</td>
<td>Oil refineries</td>
<td>12</td>
<td>0.009</td>
<td>0.023148</td>
</tr>
<tr>
<td>H032</td>
<td>Male</td>
<td>48</td>
<td>Oil refineries</td>
<td>3</td>
<td>0.004</td>
<td>0.052083</td>
</tr>
<tr>
<td>H033</td>
<td>Male</td>
<td>41</td>
<td>Oil refineries</td>
<td>11</td>
<td>0.007</td>
<td>0.029761</td>
</tr>
<tr>
<td>H034</td>
<td>Male</td>
<td>34</td>
<td>Terminals to provide fuel</td>
<td>2</td>
<td>0.007</td>
<td>0.029761</td>
</tr>
<tr>
<td>H035</td>
<td>Male</td>
<td>55</td>
<td>Terminals to provide fuel</td>
<td>35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
must be based on Student’s t test to compare the mean of iron concentration by two methods UV-visible-CPE and FAAS-CPE [31]. Table-12 shows the result of t-Test.

**Conclusion**

A sensitive, selective and environmentally friendly spectroscopic method of determination of low concentrations of Fe(III), using benzidine as a complexing agent and cloud point extraction is developed. The proposed method was successfully applied for the determination of urine samples of occupational workers.

**REFERENCES**

<table>
<thead>
<tr>
<th>Type of occupational worker</th>
<th>Type of measurement</th>
<th>d.f = n-1</th>
<th>Mean of conc. ( y \text{ (ng mL}^{-1}) )</th>
<th>Standard deviation ( \sigma_y )</th>
<th>Confidence interval of the mean ( \bar{y} \pm t_{\alpha/2, n-1} \sigma_y \sqrt{n} )</th>
<th>t-calculated ( t = \frac{\bar{y} - \mu}{\sqrt{n}} )</th>
<th>P-value</th>
<th>Type of significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil refineries</td>
<td>UV-Vis-CPE</td>
<td>13</td>
<td>0.0636</td>
<td>0.0192</td>
<td>0.0636 ± 0.0111</td>
<td>11.437</td>
<td>&lt;0.00001</td>
<td>Non-significant</td>
</tr>
<tr>
<td></td>
<td>AAS-CPE</td>
<td>13</td>
<td>0.05007</td>
<td>0.02747</td>
<td>0.0500 ± 0.0173</td>
<td>5.9283</td>
<td>0.00069</td>
<td>Non-significant</td>
</tr>
<tr>
<td>Welding</td>
<td>UV-Vis-CPE</td>
<td>11</td>
<td>0.0612</td>
<td>0.0246</td>
<td>0.0612 ± 0.0156</td>
<td>7.9277</td>
<td>&lt;0.00001</td>
<td>Non-significant</td>
</tr>
<tr>
<td></td>
<td>AAS-CPE</td>
<td>11</td>
<td>0.0481</td>
<td>0.0237</td>
<td>0.0481 ± 0.0150</td>
<td>6.3141</td>
<td>0.000058</td>
<td>Non-significant</td>
</tr>
<tr>
<td>Plumbing</td>
<td>UV-Vis-CPE</td>
<td>2</td>
<td>0.0704</td>
<td>0.0281</td>
<td>0.0704 ± 0.0095</td>
<td>4.0400</td>
<td>0.05615</td>
<td>Significant</td>
</tr>
<tr>
<td></td>
<td>AAS-CPE</td>
<td>2</td>
<td>0.0364</td>
<td>0.0090</td>
<td>0.0364 ± 0.022</td>
<td>6.0485</td>
<td>0.026266</td>
<td>Significant</td>
</tr>
<tr>
<td>Terminals to provide fuel</td>
<td>UV-Vis-CPE</td>
<td>4</td>
<td>0.0522</td>
<td>0.0236</td>
<td>0.0522 ± 0.0292</td>
<td>4.8418</td>
<td>0.019798</td>
<td>Non-significant</td>
</tr>
<tr>
<td></td>
<td>AAS-CPE</td>
<td>4</td>
<td>0.0406</td>
<td>0.0203</td>
<td>0.0406 ± 0.0252</td>
<td>3.9322</td>
<td>0.017074</td>
<td>Non-significant</td>
</tr>
<tr>
<td>Dyeing</td>
<td>UV-Vis-CPE</td>
<td>13</td>
<td>0.0849</td>
<td>0.0111</td>
<td>0.0849 ± 0.0276</td>
<td>12.4828</td>
<td>0.006357</td>
<td>Non-significant</td>
</tr>
<tr>
<td></td>
<td>AAS-CPE</td>
<td>2</td>
<td>0.0659</td>
<td>0.0334</td>
<td>0.0659 ± 0.00829</td>
<td>3.1632</td>
<td>0.080794</td>
<td>Significant</td>
</tr>
<tr>
<td>Total</td>
<td>UV-Vis-CPE</td>
<td>35</td>
<td>0.06359</td>
<td>0.0219</td>
<td>0.0635 ± 0.0073</td>
<td>16.2742</td>
<td>&lt;0.00001</td>
<td>Non-significant</td>
</tr>
<tr>
<td></td>
<td>AAS-CPE</td>
<td>35</td>
<td>0.05127</td>
<td>0.0261</td>
<td>0.0512 ± 0.0087</td>
<td>10.764</td>
<td>&lt;0.00001</td>
<td>Non-significant</td>
</tr>
</tbody>
</table>

True value \( \mu = 4.9 \text{ ng mL}^{-1} \) [Ref. 25], \( t_{0.05/2, n-1} = 2.2281 \), \( n = 3(4.303) \), \( n = 4(3.182) \), \( n = 5(2.776) \), \( n = 12(2.201) \), \( n = 14(2.160) \), \( n = 15(2.145) \), \( n = 37(2.028) \), \( n = 39(2.024) \).